

সংযোজনী: ৩

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(সর্বশেষ /চূড়ান্ত অগ্রগতি প্রতিবেদন উপস্থাপনের জন্য নির্ধারিত ছক)

১। গবেষণা প্রকল্পের শিরোনামঃ Prevalence of microbial hazards in street food and ready-to-eat salad items in restaurants and their probable risk analysis

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৩। বস্তু-সংক্ষেপঃ

Since street food and ready-to-eat salad items are an integral part of the food culture in Bangladesh. These foods, which usually do not require further processing before consumption, are widely accepted by many people in developing countries because they are viewed as nutritious, inexpensive, convenient and attractive. The World health Organization (WHO) defines street foods as ‘foods and beverages prepared and/or sold by vendors in streets and other public places for immediate consumption or consumption at a later time without further processing or preparation. This research presents a comprehensive analysis of the prevalence of microbial hazards in street food and ready-to-eat salad items served in restaurants, with particular emphasis on pathogenic bacteria (E. coli, Salmonella spp., and Vibrio spp.), in the street food including chatpoti (plate, water and tamarind chutney), cholamuri, sandwiches, sugarcane juice, and alevora sharbat with their utensils used in serving these items and restaurant salad items. The study also aims to assess the potential health risks associated with consuming these food items and provides recommendations for mitigating these risks. The presence of a higher number of harmful bacteria was found in both selected street food ready-to-eat salads and associated utensils. However, the risk analysis study found that the risk of infection from eating this item is high, and recommended that vendor’s public health awareness and access to safe water is necessary to ensure the long-term safety of these street foods.

৪। সূচনা ও পটভূমিঃ

People depend on street and restaurant foods to meet both their daily dietary needs and regular nutritional requirements because eating at home is more difficult due to their increasingly busy schedules. These delicacies from the streets and restaurants are among the most popular choices for city dwellers due to their quick availability. However, several risk factors, including sources and quality of raw foods and ingredients, food preparation, handling and vending, vending environments, vendor's hygiene practices, and the attitude of consumers to the hazards of street food, exist. However, the sector is full of harmful activities that have raised significant questions about consumers' safety and health. These unhealthy practices were found throughout the whole street food supply chain, from the agricultural raw materials to the finished retail street foods, and they have been linked to the emergence of many diseases and disorders. Scientific research has proven that residual agrochemicals in foods are detrimental to human health. Studies have shown that condiments and homemade cereal flour used in street food preparations are contaminated with *Bacillus cereus* (Gervas et al., 2005).

Environmental pollutants like airborne chemicals in dust, exhaust discharges from moving vehicles and industrial engines, burning fumes, offensive odor from accumulated waste and effluent from industrial discharge, insects, and rodents can contaminate street foods due to the conditions under which they are prepared, sold, and consumed (Ekhaton et al., 2018; Kariuki et al., 2017; Rakha et al., 2022). Airborne diseases and microbes, which may be pathogenic if allowed to settle on the prepared food surfaces, abound in the dust (Chiraporn et al., 2016). Since nearness to customers is the primary target of street food vendors, vending sites usually lack basic facilities such as toilets, hand washing facilities, potable water, good drainage, and a waste disposal system. All these conditions enhance the incidence of foodborne illnesses and transmission of diseases among vast consumers of street foods (Khairuzzaman et al., 2014). Consumers' attitudes and perceptions of hazards in street foods are often driven by their level of education, income, knowledge of food safety, age, and gender. Literature reported the various effect of these factors on consumers' attitudes to the safety of street food and their perception of hazards inherent in its consumption. Street food consumers in developing countries are generally more concerned about microbial hazards. Pathogens of significant public health importance, such as *Salmonella*, *S. aureus*, *Listeria monocytogenes*, *Campylobacter jejuni*, and *E. coli* have been isolated in some street foods in developing countries (Biswas et al., 2010; Oladipo et al., 2010; Zajeba et al., 2013). Reports in literature show that consumers know that microorganisms, especially bacteria, are responsible for foodborne diseases but have little knowledge about their pathogenesis.

In the restaurant, common vegetables utilized as salad consist of cucumber, onion slices, green chili, and sometimes tomato or carrot slices, depending on the price and season. As we know, vegetables are hardly clean and not sanitized at all from the post-harvest period to before entering into the marketing channel (Ahmed et al., 2019). After bringing the vegetables to the restaurant, the waiter washed them with drinking water, thus, posing an increased risk of microbial contamination, causing diarrhea. Many significant outbreaks reported in various continents, like Asia, Africa, and South America, could arise from consuming contaminated salads (WHO, 2002; Meldrum et al., 2009; Adjrah et al., 2013). The microbial load and the presence of bacterial pathogens in foods are a good indication of the food quality and the potential health risk they pose to consumers (Ahmed et al., 2019). Thus, studies must be undertaken to understand the magnitude of microbial hazards and their risk analysis for causing disease upon consuming this contaminated food.

Risk analysis is a tool that has been developed for safe food production by reducing food-related illnesses (Collado et al., 2011). Regulatory authorities and food processors use this tool to control microbial hazards to ensure consumers' microbiologically safe and sound foods (Duffy et al., 2006). Among the significant elements of risk analysis, the risk assessment employs scientific and statistical information to estimate the likelihood and severity of illness or death (Duffy et al., 2006; Cassin et al., 1998). For the identification of the microbial risks linked with the utilization of specific food, Quantitative microbial risk assessment (QMRA) is beneficial and also provides measures of the level of infection caused by a pathogen in a particular population consumed a specific food (Forsythe, 2002). This ensures that the resources are purposefully coordinated to help limit the risk brought about by foodborne microorganisms.

Objectives: This research aims to determine the presence of pathogenic bacteria, with particular emphasis on *E. coli*, *Salmonella* spp., and *Staphylococcus* spp., in RTE street food and restaurant salad vegetables and the risk of consuming this food by predictive model (exponential) using Monte Carlo simulation

Problem Analysis

The food habits of Bangladeshi consumers have changed remarkably in recent years due to rapid urbanization, economic growth, and women empowerment, which changed people's lifestyles. The involvement of women in official jobs has changed the family structure, encouraging more people to consume ready-to-eat food or eat out in a restaurant compared to home-cooked food, which was the

standard practice in the last century. People from low and middle-socioeconomic status and students are the most popular consumers of street and restaurant foods (Biswas et al., 2010). Street foods are prepared and stored in a contaminated environment, and most of the time, street foods are not covered and are exposed to flies and dust, which can contribute to foodborne diseases upon consumption (WHO, 2002). Contamination of street foods persists through preparation and cooking due to the quality of raw materials and post-cooking handling due to poor personal hygiene (Rane, 2011). Lack of potable water for various activities, vendors, reuse the water for cleaning utensils and used dishes. In addition, street vendors' low educational qualifications, socioeconomic status, lack of knowledge of safe food handling, mobility, diversity, and temporary nature also contribute to public health risks. Scientific research has proven that residual agrochemicals in foods are detrimental to human health. However, the methods and practices used in salad preparation, handling, and sale make them vulnerable to cross-contamination, recontamination, and the transfer of pathogens and foodborne diseases. Mode of transportation significantly contributes to the contamination of agricultural products. It has been reported that transportation and holding temperature play a significant role in accelerating their spoilage and transmission of zoonotic diseases. The long holding period of more than 6 h, sometimes at ambient temperature, was reported to be a common factor contributing to foodborne illness through the multiplication of microorganisms favored by holding temperatures in the range of 5 and 60°C (described as danger zone). Most salad dishes served in restaurants are typically made in large quantities ahead of time at various periods. Furthermore, while holding salad dishes, incessant uncovered may be exposed to dust contamination and flies, which has been linked to foodborne diseases such as cholera and diarrhea. Hence this study aimed to analyze the prevalence of bacterial pathogens in street food and ready-to-eat salad items in restaurants and their probable risk analysis.

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METHODS & MATERIALS

6.1. Sample collection:

Street food samples including chatpoti (plate, water and tamarind chutney), cholamuri, sandwiches, sugarcane juice, alevora sharbat and restaurant salad items from 25 sampling site (two adjacent thana is considered as one sampling area) of Dhaka City Corporation area will be collected. The vendors/ restaurant sites will be selected systematically, considering the area's stall structure, location, and

population density. Three vendors/restaurant sites was randomly chosen from each selected thana, located at least 1 km from one another. Thus, 6 types x 3 x 25=450 samples will be collected from Dhaka City Corporation area. Thus, 450 samples was analyzed, and the vendors'/restaurants' site hygiene and salubrious status was determined through short structured interviews and observations. Each sample was collected during site visits in a pre-sterilized container or sample bag. The vendor was asked to pour the samples into the sample bag without touching it. The sample containing bags was placed in an icebox and transported to FNARL, where it was coded and sent for analysis. Each sample's microbiological quality and safety was analyzed following cultural and molecular approaches.

6.2. Microbiological analysis:

Briefly, twenty-five (25) g of each sample will be homogenized in 225 milliliters of saline water (0.85% NaCl). Decimal dilutions will be prepared upto 10^{-6} , and appropriate dilutions will be spread plated on both selective and non-selective agar medium followed by incubation at 35°C for 24-48 hours before being counted. Tryptic Soy Agar (TSA) and Sorbitol MacConkey Agar (SMAC) (Oxoid Ltd., Hampshire, England) will be used for the total aerobic bacterial count and coliform count. Eosine Methylene Blue (EMB) agar media (Nissui Co., Ltd., Tokyo, Japan) for *E. coli*, Bismuth sulfide Agar (BSA) for *Salmonella* spp, TCBS Agar for *Vibrio* spp Five typical random colonies from each plate will be collected, and API diagnostic kits will do presumptive confirmation.

6.3. Microbial risk analysis:

Microbial risk analysis of food involves four steps: hazard identification, hazard characterization, exposure assessment, and risk characterization (CAC, 2010). In order to assess microbial risk, this study focused on the risk of street food and salad consumption that are available and sold in the Dhaka city area.

a) Hazard identification: Microbiological risk assessment starts with hazard identification. The purpose of hazard identification was to identify the microorganism (s) present in RTE street food and salad samples. Three organisms, *Vibrio* spp., *Salmonella* spp., and *E. coli*, were selected as the model QRMA organisms as they are frequently found in street food and salads samples.

b) Hazard characterization (dose-response assessment): Hazard characterization defines the adverse health effect related to the consumption of microorganisms. An exponential dose-response model (Rose and Haas, 1999) was used for dose-response assessment to predict the probability of *Salmonella* spp.,

Vibrio spp., and *E. coli* infection based on laboratory output of selected microorganisms. Mathematically, the equation used for determining probability is illustrated as,

$$P(d) = 1 - e^{-rd} \quad (1)$$

Where, $P(d)$ indicates the probability of infection, d denotes the dose (CFU) of microorganisms consumed per person per day, and r refers to the dimensionless infectivity constant. The model parameter " r " used was 1×10^{-6} for *Vibrio* Spp. (Mahendra and Uma, 2007), 1×10^{-5} for *Salmonella* spp. (Mahendra and Uma, 2007), and 1×10^{-5} for *E. coli* (Mahendra and Uma, 2007).

The final dose (d) of microorganisms consumed per person per serving was the product of the weight of street food or salad consumed and the number of organisms present per gram of street food or salad. The final doses of *Vibrio* spp., *Salmonella* spp., and *E. coli* consumed per serving were inputted into the mathematical model to obtain the probability of infection per day.

c) Exposure assessment: This step deals with the amount of food consumed and the frequency of consumption of food for a specified period to assess the consumer's exposure to microorganisms with specific food samples for a defined time. The volume of food consumed must be quantified in order to estimate the dose of pathogens, where only exposure by oral ingestion was considered. The volume of food consumed (grams per day) is multiplied by the pathogen concentration at the serving plate to calculate the total exposure or dose (consumed dose = $\mu \times V$) per day

d) Risk characterization: This step links the previous three steps and estimates the probability of infection resulting from exposure to hazards in every exposure scenario. The annual infection probability for each situation was estimated from the probability of infections calculated from the number of days consumed within the year.

$$P_{\text{ann}} = 1 - [1 - P(d)]^n \quad (2)$$

Where, n denotes the number of days of exposure within the year.

6.4. Statistical analysis: All the data were incorporated in Statistical Package for the Social Sciences 25.0 (IBM Corporation, USA) for descriptive analysis. Experimental data were analyzed by one-way analysis of variance, and appropriate and significant differences among different restaurants were made at 95% confidence level ($p < 0.05$) by Tukey's multiple comparison test (Zar 1999). For each exposure scenario, the Monte Carlo simulation was run using Microsoft excel@risk software version 7.5.0 (Palisade Corporation), sampling 10,000 iterations to measure the annual risk of infection

RESULTS AND DISCUSSION**7.1.CHOLAMURI:**

On an average, 6.56 ± 0.06 log CFU/g of total aerobic bacterial, 5.84 ± 0.27 log CFU/g of total coliform counts and 4.18 ± 0.13 log CFU/g of total fungal count were recorded in **Cholamuri** samples. Presence of higher number of total aerobic bacteria indicate that the **Cholamuri** is of low quality, on the other hand, presence of higher number of total coliform bacteria indicate that the **Cholamuri** was prepared in unhygienic environmental condition and presence of higher number of yeast & mold count indicates the inadequately sanitized equipment or as air borne contaminants. All these microbial counts were found beyond the permissible limit of the street food (Table 1). In addition, higher number of *Escherichia coli* (3.57 ± 0.10 CFU/g) was evident in **Cholamuri** sample indicates that the cholamuri samples were contaminated with fecal materials. Furthermore, 3 **Cholamuri** samples of 46 samples (6.5%) were found contaminated with *Salmonella* spp. indicating the presence of pathogens that could pose significant health risk of consumers. This finding suggested that the **Cholamuri** samples collected sold in Dhaka city were not comply with the food safety standard and may pose significant health risk of consumers.

Vibrio spp. was evident in all the samples, with an average count of 2.88 ± 0.11 log CFU/g, which may enter food products through contamination of equipment and instruments by growing biofilms on inert surfaces.

Table 1: Average microbiological quality of Cholamuri sold in Dhaka city (n=75)

Parameters Analyzed	Average microbial population (log CFU/g) in Cholamuri samples			ICMSF permissible limit
	Average	Highest	Lowest	Standard value
Total aerobic bacteria	6.56 ± 0.06	7.48	4.9	4.0-5.0 log CFU/g
Total coliform bacteria	5.84 ± 0.27	7.28	3.81	Max 2.0 log CFU/g
Total yeast & mold count	4.18 ± 0.13	5.65	2.6	Max 3.0 log CFU/g
<i>Escherichia coli</i>	3.57 ± 0.10 (68)	5.48	A (7)	A
<i>Salmonella</i>	P (3)	-	A (72)	A
<i>Vibrio</i> spp	2.88 ± 0.11 (32)	3.57	2.19	Not Mentioned
Average pH	5.8	6.8	5.0	Not Mentioned

*P= Present after enrichment; A= Absent even after enrichment; Letters within the parenthesis indicate number of samples

In our previous study done with Jhal muri samples in FY 2017-2018, total aerobic bacterial count was recorded as 3.2×10^7 CFU/g and the total coliform bacteria was recorded as 1.2×10^5 CFU/g in Jhal muri samples. Both of these values are higher than the recommended standard. However, no pathogenic *E. coli* O157, O111 or O26, nor non-pathogenic *E. coli* were observed in all the Jhal muri samples. On the other hand, other foodborne pathogen including *Salmonella* spp., *C. Sakazakii*, *Yersinia* spp., *Staphylococcus* spp., *Enterococcus* spp., *Listeria monocytogenes* was observed in all the samples tested and spoilage microorganisms *Bacillus* spp., *Pseudomonas* spp., and LAB were also found in all the samples. Visual observation and collected questionnaires data reveal that most of the Jhal Muri vendor has no formal education, no formal training on hygiene, no license nor any water or washing materials to wash the utensils or hand. He used to prepare Jhal Muri constantly using same container without any wash. These vendors did not use hand gloves, mask or hair-net, instead used his open hand to prepare and served everything and even took money with the same hand. Therefore, every chances of cross contamination with microorganisms can be occurred during handling and preparation.

7.2. SANDWICHES

The total aerobic bacterial count in sandwich samples were recorded as 6.85 ± 0.12 log CFU/g, total coliform count was recorded as 5.04 ± 0.14 log CFU/g, and total faecal coliform was recorded as 4.47 ± 0.71 log CFU/g. The total yeast and mold count was recorded as 5.17 ± 0.61 log CFU/g. Although no *E. coli* and *Salmonella* spp was detected in the plate count methods but after enrichment *E. coli* was found in 6 (8.0%) samples and *Salmonella* was detected in 3(4.0%) samples. However, *Vibrio* spp contamination was recorded in all the sandwich samples, with an average *Vibrio* spp count of 4.90 ± 0.31 log CFU/g (**Table-2**).

Table 2: Average microbiological quality of **Sandwiches** sold in Dhaka city (n=75)

Parameters Analyzed	Average microbial population (log CFU/g) in Sandwiches			ICMSF permissible limit
	Average	Highest	Lowest	Standard value
Total aerobic bacteria	6.85 ± 0.12	7.13	6.57	4.0-5.0 log CFU/g
Total coliform bacteria	5.04 ± 0.14	6.28	3.81	Max 2.0 log CFU/g
Total fecal coliform count	4.47 ± 0.71	5.51	3.43	Max 1.0 log CFU/g
Total yeast & mold count	5.17 ± 0.61	5.89	4.45	Max 3.0 log CFU/g
<i>Escherichia coli</i>	<1.0; P(6)	-	A (69)	A
<i>Salmonella</i>	<1.0; P (3)	-	A (72)	A
<i>Vibrio spp</i>	4.90 ± 0.31 (22)	5.15	4.65	not mentioned

*P= Present after enrichment; A= Absent; Letters within the parenthesis indicate number of samples

The overall aerobic bacterial count was determined to be greater than the ICMSF acceptable limit, indicating a shortened shelf life for the sandwiches. A higher coliform count suggests an unsanitary situation in the sandwich preparation area. Higher fecal coliform counts indicated the presence of fecal elements in the sandwiches. On the other hand, the presence of *E. coli* and *Salmonella* in sandwiches indicates fecal contamination of the product. The presence of *Vibrio* spp. indicates contaminated water or undercooked food components used in sandwich production. *Vibrio* spp. enter food products through contamination of equipment and instruments by growing biofilms on inert surfaces. However, as the sandwiches are usually wrapped with cellophane paper, thus, chances of cross-contamination after preparation is negligible, however, abuse of holding temperature and time may increase the bacterial count, and can cause significant health problems.

The microbiological status of the different types of sandwiches e.g. egg salad sandwiches, chicken salad sandwich, beef sandwich and vegetable sandwich from various fast food shops of Dhaka city was done by Trina Hasan in 2014 and observed that the sandwich sample contains *Alcaligenes* spp., (2.7%) *Bacillus* spp., (2.7%), *Shigella* spp, (2.7%), *Klebsiella* spp., (5.4%), *Enterobacter* spp., (10.5%), *Escherichia coli* (10.8%) and *Salmonella* spp., (10.8%), *Vibrio* spp., (13.5%), *Staphylococcus* spp., (19%) and *Saccharomyces* spp (13.5%) depending on the types of the sandwich. The highest aerobic bacterial count was recorded in beef sandwich as 8.78 ± 0.22 log CFU/g, chicken salad sandwich as 6.57 ± 0.31 log CFU/g, egg salad sandwich as 6.32 ± 0.22 log CFU/g) and vegetable sandwich as 4.36 ± 0.17 log CFU/mg). Similar experimental results were obtained by N. Hoque et al.2012. The degree of initial contamination in sandwiches was higher in all cases, posing a risk to public health. It was concluded that the preservation of personal cleanliness and process hygiene when producing sandwiches may preserve the quality and safety features required for consumer acceptability and safety.

7.3. CHATPATI:

On an average, 6.61 ± 0.73 log CFU/g of total aerobic bacterial, 5.71 ± 1.17 log CFU/g of total coliform counts and 4.14 ± 0.67 log CFU/g of total fungal count were recorded in Chatpatati samples. Presence of higher number of total aerobic bacteria indicate that the Chatpatati is of low quality, on the other hand, presence of higher number of total coliform bacteria indicate that the Chatpatati was prepared in unhygienic environmental condition. Furthermore, presence of higher number of yeast & mold count indicates the inadequately sanitized equipment or as air borne contaminants. All these microbial counts were found far beyond the permissible limit of the street food (Table 4). In addition, higher number of

Escherichia coli (3.56 ± 1.62 CFU/g) was evident in Chatpati sample indicating that the Chatpati samples were somehow contaminated with faecal materials. The microbiological analysis data revealed that the chatpati samples collected from the Dhaka city were not comply with the food safety standard and may pose significant health risk of consumers.

Table 3: Average microbiological quality of Chatpati sold in Dhaka city (n=75)

Parameters Analyzed	Average microbial population (log CFU/g)			ICMSF permissible limit
	Average	Highest	Lowest	Standard Value
Total aerobic bacteria	6.61 ± 0.73	7.56	5.28	4-5 log CFU/g
Total coliform bacteria	5.71 ± 1.17	7.51	2.3	Max 2.0 log CFU/g
Total yeast & mold count	4.14 ± 0.67	5.97	3.3	Max 3.0 log CFU/g
<i>Escherichia coli</i>	3.56 ± 1.62 (72)	5.48	A (3)	A
<i>Salmonella</i>	A (75)	-	A (75)	A
<i>Vibrio spp</i>	4.18 ± 0.48 (35)	5.14	3.23	Not mentioned
Average pH	6.11	7.8	3.2	Not Mentioned

*P= Present after enrichment; A= Absent; Letters within the parenthesis indicate number of samples

In our previous study done with chatpati samples in FY 2017-2018, total average aerobic bacterial count was recorded as 1.1×10^8 CFU/g and the total coliform bacteria was recorded as 7.1×10^5 CFU/g in chatpati samples. Both of these values are higher than the recommended standard. However, no pathogenic *E. coli* O157, O111 or O26, nor non-pathogenic *E. coli* were observed in all the Chatpoti samples. On the other hand, other foodborne pathogen including *Salmonella* spp., *C. Sakazakii*, *Yersinia* spp., *Staphylococcus* spp., *Enterococcus* spp., *Listeria monocytogenes* was observed in all the samples tested and spoilage microorganisms including *Bacillus* spp., *Pseudomonas* spp., and LAB were also found in all the samples. On the other hand, visual observation and collected questionnaires data reveal that most of the vendors boiled yellow chickpeas and potatoes in previous night and vended it from morning to evening and kept in open container. However, the other ingredients including vegetables slices were made 1 hours before and kept on either mix or separate container. The vendors did not wear gloves while slicing the pieces of vegetables or while preparation and mixing the chatpoti with the ingredients. Moreover, the vendors uses his open hand constantly to prepare chatpoti and served to 100 of customers in a day. It was also observed that chotpotti vendors use a piece of cotton to wipe his hand if needed (no wash in

between). The spoon served with plate was rinsed in previously used water in a pot. Therefore, almost all the hazard is creating by this vendor through his activities. To overcome this situation, a good hygiene practice of the street food vendors is necessary.

7.4. SUGARCANE JUICE

The average pH of sugarcane juice was recorded as 5.33. The total aerobic bacterial count (TABC) in sugarcane juice samples was recorded as 6.94 ± 0.15 log CFU/ml, coliform count as 5.34 ± 0.24 log CFU/ml, total faecal coliform count as 3.69 ± 0.89 log CFU/g, and total yeast and mold count was recorded as 4.74 ± 0.82 log CFU/ml. The presence of *E. coli* was evident in 67 (89.3%) sugarcane juice samples with an average count of 2.40 ± 0.03 log CFU/ml, and *Salmonella* and *Vibrio* spp was evident in 52(69.3%) and 22 (29.3%) samples, with an average count of 1.85 ± 0.22 and 1.71 ± 0.04 log CFU/ml, respectively (Table-4). The Mug used to collect the sugarcane juice and the glass used to serve sugarcane juice was found grossly contaminated with *E.coli*, *Salmonella* and *Vibrio* spp. About 60 (80.0%) Mug, 52(69.3%) glass were found contaminated with *E.coli* and these values for *Salmonella* was 52(69.3%) and 20(16.6%), respectively. *Vibrio* spp contamination in Mug and glass was recorded as 45 (60.0%) and 52(69.3%), respectively (Table-4). In addition, total aerobic bacterial count (5.06 ± 0.40 log CFU/swab), total coliform bacterial count (2.70 ± 0.04 log CFU/swab), total fecal coliform bacterial count (1.68 ± 0.02 log CFU/swab) and total yeast and mold count (4.63 ± 0.35 log CFU/swab) was also found high in Mug and these values for glass was recorded as 5.21 ± 0.38 , 3.59 ± 0.18 , 2.88 ± 0.13 and 4.26 ± 0.05 log CFU/swab, respectively, meaning that the mug or the glass was not clean and sanitized properly.

Table-4: Average microbiological contamination in sugarcane juice, glass to serve and mug to prepare sugarcane juice, collected from various areas of Dhaka city (n=75).

Test organisms	Average microbial population (log CFU/ml)		
	Sugarcane juice	Glass	Mug
Total aerobic bacteria	6.94 ± 0.15 (75)	5.21 ± 0.38 (75)	5.06 ± 0.40 (75)
Total coliform bacteria	5.11 ± 0.01 (75)	3.59 ± 0.18 (67)	2.70 ± 0.04 (75)
Total fecal coliform bacteria	4.59 ± 0.30 (75)	2.88 ± 0.13 (52)	1.68 ± 0.02 (75)
Total yeast & mold count	5.26 ± 0.14 (75)	4.26 ± 0.05 (75)	4.63 ± 0.35 (75)
<i>Escherichia coli</i>	2.40 ± 0.03 (67)	<1.0; P (52)	<1.0; P (60)
<i>Salmonella</i>	1.85 ± 0.22 (52)	<1.0; P (20)	<1.0; P (52)
<i>Vibrio spp</i>	1.71 ± 0.04 (22)	<1.0; P (52)	<1.0; P (45)

*The average values of each individual trial \pm SD; <1.0=not detected; P=Present after enrichment; Letters within the parenthesis indicate number of samples

The microbiological investigation results showed that the sugarcane juice, glass, and mug were significantly contaminated with fecal coliform, *E. coli*, *Salmonella*, and *Vibrio* spp. This contamination could be caused by the use of unclean sugarcane stalks and pressing machines, unclean utensils, and contaminated water. The vendor's filthy hands and personal hygiene might also contribute to contamination. In a study done by Khan et al., 2015 reported an average total viable count (microbial load) and total coliform counts ranging from $7.7 \times 10^3 - 9 \times 10^8$ cfu/ml and $2.1 \times 10^2 - 1.1 \times 10^3$ CFU/100 ml, was recorded in sugarcane juice. Various pathogenic species of bacteria such as *Proteus* spp., *Enterobacter* spp, *E. coli*, *Shigella* spp, *Citrobacter* spp, *Vibrio* spp, *Yersinia* spp and *Hafnia* spp were isolated from the juices. They found that unhygienic water for dilution, dressing with ice, prolonged use without refrigeration, insanitary surroundings, raw materials, chemical properties, equipment, fruit flies and airborne dust are the contributory factors of contamination.

Another study conducted by Md. Mahmudul Hasan Polin in 2018, showed that sugarcane juice sold in Dhaka City Street was heavily contaminated with pathogenic microorganisms. The total aerobic bacterial count was recorded ranging from 2.5×10^8 to 3.7×10^9 CFU/ml, and total coliform count ranging from 5×10^6 to 4×10^7 CFU/ml. *E. coli* was also found and the count ranging from 2.0×10^4 to 3.6×10^4 CFU/ml, *Salmonella* and *Shigella* spp were also observed in the sugarcane juice samples, and the counts ranging from 5.2×10^2 to 1.7×10^4 CFU/ml and 15×10^3 to 1.2×10^5 CFU/ml, respectively.

7.5. ALOE VERA SHARBAT

The average TABC in aloe vera sharbat samples were recorded as 6.88 ± 0.27 log CFU/ml, total coliform count was 5.47 ± 0.23 log CFU/ml, total fecal coliform count was 4.98 ± 0.46 log CFU/ml, and total yeast & mold count was recorded as 4.14 ± 0.51 log CFU/ml. The presence of *E. coli* was evident in 72(96.0%), with an average count 2.35 ± 0.14 log CFU/ml and *Salmonella* in 16 (21.3 %), with an average count of 3.87 ± 0.40 log CFU/ml in aloe vera sharbat samples. The *Vibrio* spp was evident in 64 (85.3) with an average count of 4.14 ± 0.21 log CFU/ml and *Listeria* spp was found in 7 (9.3%), with an average count of 2.80 ± 0.09 log CFU/ml, aloe vera sharbat samples. (Table-5). The Mug used to prepare aloe vera sharbat and the glass used to serve aloe vera sharbat was found grossly contaminated with *E. coli*, *Salmonella* and *Vibrio* spp. About 61 (81.3%) Mug, with an average count of 2.67 ± 0.41 log CFU/swab and 56(74.6%) glass, with an average count of 2.39 ± 0.26 log CFU/swab were found contaminated with *E. coli* and these values for *Salmonella* was 17(22.6%), with an average count of 2.87 ± 0.40 log CFU/swab and 16 (21.3%), with an average count of 3.77 ± 0.08 log CFU/swab, respectively. *Vibrio* spp contamination in mug and glass was

recorded as 54 (72.0%), and 45 (60.0%), respectively and *Listeria* spp contamination in Mug and Glass was recorded as 4 (5.3%) and 7 (9.3%), respectively. (Table-5). In addition, total aerobic bacterial count (4.90 ± 0.20 log CFU/swab), total coliform bacterial count (3.63 ± 0.34 log CFU/swab), total fecal coliform bacterial count (3.02 ± 0.26 log CFU/swab) and total yeast and mold count (3.14 ± 0.27 log CFU/swab) was also found high in Mug and these values for glass was recorded as 4.48 ± 0.22 , 3.12 ± 0.53 , 2.32 ± 0.22 and 2.10 ± 0.35 log CFU/swab, respectively, meaning that the mug or the glass was not clean and sanitized properly.

Table: 5: Average microbiological contamination in aloe vera juice, glass to serve and mug to prepare aloe vera juice, collected from various areas of Dhaka city (n=75).

Test organisms	Microbial population log CFU/ml (number of sample positive)		
	Aleovera Juice	Glass	Mug
TABC	6.88 ± 0.27 (75)	4.48 ± 0.22 (75)	4.90 ± 0.20 (75)
TCC	5.47 ± 0.23 (75)	3.12 ± 0.53 (75)	3.63 ± 0.34 (75)
TFCC	4.98 ± 0.46 (75)	2.32 ± 0.22 (75)	3.02 ± 0.26 (72)
Y & M	4.14 ± 0.51 (75)	2.10 ± 0.35 (58)	3.14 ± 0.27 (68)
<i>E. coli</i>	2.35 ± 0.14 (72)	2.39 ± 0.26 (56)	2.67 ± 0.41 (61)
<i>Salmonella</i> spp	3.87 ± 0.40 (16)	3.77 ± 0.08 (16)	2.87 ± 0.40 (17)
<i>Vibrio</i> spp	4.14 ± 0.21 (64)	1.01 ± 0.02 (45)	2.70 ± 0.11 (54)
<i>Listeria</i> spp	2.80 ± 0.09 (7)	<1.0; P (7)	<1.0; P (4)

*The average values of each individual trial \pm SD; <1.0=not detected; P=Present after enrichment; Letters within the parenthesis indicate number of samples

7.6. MIXED SALAD

The total aerobic bacterial count in mix salad samples was recorded as 5.31 ± 0.24 log CFU/g and total coliform count was recorded as 2.53 ± 0.18 log CFU/g. *E. coli* was evident in 38 (50.6%) samples, with an average count of 1.78 ± 0.09 log CFU/g, and *Salmonella* spp was recorded in 12 (16.0%) samples, with an average count of 1.22 ± 0.15 . The presence of *Vibrio* spp was recorded in 27 (36.0%) samples only after enrichment (**Table-6**). Since the presence of *E.coli* and *Salmonella* spp was evident in 50.6% and 16% meaning that these vegetables are somehow contaminated with fecal materials, which may cause diarrhea upon consumption.

Table-6: Average microbiological quality of mixed salad vegetables sold in restaurants of Dhaka city.

Type of Samples	Average microbiological population (log ₁₀ CFU/g)				
	Total aerobic bacterial count	Total coliform count	<i>E. coli</i>	<i>Salmonella</i> spp.	<i>Vibrio</i> spp
Mixed salad (n=75)	5.31 ± 0.24 (75)	2.53± 0.18 (72)	1.78 ± 0.09 (38)	1.22 ± 0.15 (12)	<1.0; P (27)

*The average values of each individual trial ±SD; <1.0=not detected

n= Total number of samples; P=Present after enrichment; Letters within the parenthesis indicate number of samples.

In our previous study done in between 2017-2019, we observed similar microbiological contaminations in fresh raw salad vegetables, including tomato, cucumber, lettuce, green chilli and coriander leaf. The presence of foodborne pathogens including *E. coli*, and *Salmonella* spp. was evident in almost all the salad vegetables samples (Ahmed et al., 2019). As, vegetables are hardly clean, and not sanitized at all before entering into the marketing channel in Bangladesh that contribute unsafe and poor-quality vegetables, therefore, cleaning and sanitation practices must be introduced to improve the quality and safety of these vegetables. In addition, number of factors including 1) lack basic knowledge and awareness in safe handling practices during production and post-harvest operations; 2) inadequacy of postharvest specific infrastructure such packing houses; 3) pre-cooling, sorting and storage facilities; 4) lack of auxiliary industries for the production of packaging materials, tools and equipment; 5) deterioration of produce quality owing to rough handling, improper packaging; 6) overloading and damage during transportation; 7) lack of cold chain systems; 8) unskilled farmers coupled with poor technical extension and training facilities, and 9) poor access to market information, were found responsible for the poor quality and unsafe vegetables. Thus, introduction of non-chlorine sanitizers for washing, along with improvement of the above-mentioned factors should be taken care to improve the safety and quality of raw salad vegetables.

7.7. QUANTITATIVE MICROBIOLOGICAL RISK ASSESSMENT

The street food examined in the quantitative microbiological risk assessment study was classified into three categories: 1) Street snacks include cholamuri, chatpoti, and sandwiches; 2) beverages include juice and sherbet; and 3) restaurant salads are classified as fresh vegetables.

a) Hazard identification: Microorganisms that produce negative health consequences are typically identified during the hazard identification process. The presence of microorganisms indicating faecal contamination is a strong indicator and a risk to public health. For this study, *E. coli*, *Salmonella*, and *Vibrio* spp., were chosen as foodborne pathogens capable of causing diseases in humans. Thus, for all three categories of food in this investigation, *E. coli*, *Salmonella*, and *Vibrio* spp alone or in combination is regarded an index hazard in accordance with the referred works (Schmid-Hempel and Frank, 2007; Haas et al., 2014; Carducci et al., 2020). The presence of *E. coli*, *Salmonella*, and *Vibrio* spp., either alone or in combination, in any ready-to-eat food category indicates a poor function of one or more system controls, as well as a pathway for fecal contamination to potentially reach the consumer, which is unacceptable. As a result, the regulatory guideline recommended for *E. coli*, *Salmonella*, and *Vibrio* spp in ready-to-eat food is a maximum allowable concentration of none detectable per 25 g in ready to eat food.

Dose-response assessment: When assessing the exposure of pathogens to food, both the concentration of pathogens in food and volume of food consumed are important parameters. In this study, for snacks category ready to eat cholamuri, chatpoti and sandwiches, for cholamuri 75g/serving, Chatpoti 225 g/ serving and sandwich maximum 200 g per /serving was found. For beverages category, sugarcane juice 250 ml/glass; and aloe vera sharbat 250 ml/glass was recorded. For fresh vegetable category, mixed salad maximum 25g/serving. The average dose of ingested pathogens obtained by multiplying the consumed volume of food per serving by the recorded average value of each pathogens. Since 8% of the total *E. coli* is considered pathogenic, the dose was multiplied by 0.08 (WHO, 2016; Daley et al., 2019; Carducci et al., 2020) and were presented in Table 7.

Table 7: Average dose of pathogenic bacteria ingested after each serving of street foods.

Street food items	<i>E.coli</i> CFU/serving	<i>Salmonella</i> CFU/serving	<i>Vibrio</i> spp CFU/serving
Cholamuri	5.8×10^6	7.5×10^2	7.5×10^2
Chatpoti	5.9×10^4	2.0×10^3	3.0×10^6
Sandwiches	1.6×10^2	2.0×10^3	1.6×10^7
Sugercane Juice	5.2×10^3	1.7×10^4	1.3×10^4
Aloe vera Sharbat	4.5×10^3	1.8×10^6	1.4×10^4
Mix salad	1.4×10^2	5.1×10^2	3.0×10^2

The probability of infection per day (P_{inf}) was calculated using Eq. 1

$$P(d) = 1 - e^{-rd} \quad (1)$$

Where, $P(d)$ indicates the probability of infection, d denotes the dose (CFU) of microorganisms consumed per person per day, and r refers to the dimensionless infectivity constant. The model parameter " r " used was 1×10^{-6} for *Vibrio Spp.* (Mahendra and Uma, 2007), 1×10^{-5} for *Salmonella spp.* (Mahendra and Uma, 2007), and 1×10^{-5} for *E. coli* (Mahendra and Uma, 2007).

Therefore the probability of infection by consuming cholamuri = $1 - e^{-(5.8 \times 10^6)(1 \times 10^{-5})} = 1 - e^{-5800000 \times 0.00001} = 1 - e^{-58} = 1 - \infty = 1.0$ per serving, We assume that people consume once in a day which indicates the probability of infection due to the consumption of *E. coli* through cholamuri is=1.0. Since *E.coli* contamination was observed in 68 samples (90.6%) samples, thus the probability of infection is= $0.60 \times 90.6\% = 0.91$
Likewise, similar calculation was done for other foods contaminated with specific pathogens and presented in table 8.

Table 8: The probability of infection after consuming each pathogen through street foods.

Pathogen	Probability of infection (P_{inf}) after consuming					
	Cholamuri	chatpoti	sandwiches	sugarcane juice	Aloevera sharbat	mixed salad
<i>E.coli</i>	0.91	0.42	0.0	0.04	0.04	0.0
<i>Salmonella spp</i>	0.0003	0.0	0.001	0.11	0.21	0.16
<i>Vibrio spp</i>	0.0003	0.44	0.29	0.004	0.012	0.0001

Exposure assessment: This step deals with the amount of food consumed and the frequency of consumption of food for a specified period to assess the consumer's exposure to microorganisms with specific food samples for a defined time. The volume of food consumed must be quantified in order to estimate the dose of pathogens, where only exposure by oral ingestion was considered. The volume of food consumed (grams per day) is multiplied by the pathogen concentration at the serving plate to calculate the total exposure or dose ($consumed\ dose = \mu \times V$) per day as shown in Table 7.

Risk characterization

This step links the previous three steps and estimates the probability of infection resulting from exposure to hazards in every exposure scenario. The annual infection probability for each situation was estimated from the probability of infections calculated from the number of days consumed within the year.

$$P_{\text{ann}} = 1 - [1 - P(d)]^n \quad (2)$$

Where, n denotes the number of days of exposure within the year. For cholamuri consumption, the probability of infection value with *E.coli* was recorded as 0.91 meaning that 91 people out of 100 people can be infected (Table 8). This study assumes that for any ingested foodborne pathogens, the resistance of any human host to infections is mathematically uncertain and is a probability between 0 and 1. The study therefore presumes the infectivity parameter, to be 0.5, as a 50% chance that a pathogen will survive to initiate an infection once ingested by a human host. The infectivity parameter as 0.5, which is a middle course probability between no risk (0) scenario and worst risk scenario. Thus, 50% of 91 people out of 100 people will be infected per consumption. If we assume that people consume cholamuri once in a week, then $45.5 \times 52 = 2,366$ out of 5200 people will be infected annually. Therefore, the risk assessment study findings revealed that the highest possibility of *E.coli* infection is from cholamuri (45.5%) followed by Chatpoti (21.0%), sugarcane juice (4.0%), aloe vera sharbat (4.0%), and negligible risk from mixed salad (0.00%) and sandwiches (0.00%).

The highest probability of infection with *Salmonella* is from aloe vera sharbat (10.5%), followed by mixed salad (8.0%), and sugarcane juice (5.5%), while minimal or no risk of infection associated with *Salmonella* from cholamuri, sandwiches and chatpoti.

On the other hand, the highest probability of infection with *Vibrio* is from chatpoti (22.0%) followed by sandwiches (14.5%), while minimal risk of infection associated with vibrio is from aloe vera sharbat, sugarcane juice, mixed salad and cholamuri.

It's crucial to realize that infection and illness are not synonymous. People who become infected with a pathogen do not necessarily feel sick or exhibit symptoms. Many people become infected with pathogens despite having no symptoms and according to WHO, the method for calculating the risk of illness is to divide the risk of infection by two or another relevant factor. If the expected chance of infection is 0.46, the risk of sickness is approximately 0.23. Another way to look at it is that if 100 persons were exposed to these bacteria, approximately 46 of them would become infected, whereas 23 would become ill. Because the bacterial risk of illness is equal to the ingested dose of pathogens on the day, yearly illness should be read as the frequency of consuming contaminated food.

Limitation of this study:

We assessed the chance of infection based on our findings, but we estimated the probability of infection based on the work of other researchers because we lack a nationwide database of infectivity dose and illness. According to WHO, the method for calculating the risk of illness is to divide the risk of infection by two or another relevant factors. If the expected chance of infection is 0.42, the risk of sickness is approximately 0.21. Another way to look at it is that if 100 persons were exposed to these viruses, approximately 42 of them would become infected, whereas 21 would become ill. Because the bacterial risk of illness is equal to the ingested dose of pathogens on the day, yearly illness should be read as the frequency of consuming these contaminated food.

Recommendation

This kind of surveillance research can provide a basic scientific information required for taking actions to improve food hygiene conditions by implementing suitable food hygienic measures by the governments. This finding of this study suggesting improvement of food handler's knowledge through training in reducing foodborne disease incidence throughout the country, and much active communication and collaboration among stakeholders / Government / NGOs, and laboratories will enhance dealing with the global food safety issues.

Major Observations

1. Due to limited access to safe water, street food vendors lacked proper hygiene and sanitation measures. Hygienic techniques in food preparation, utensil handling during serving, vendor personal cleanliness, and methods for keeping cooked food while selling throughout the day.
2. Given that street food vendors operate without a license, it's crucial to provide them with clear legal standing so they can collect their entitlements and sustain their livelihood.
3. Due to a lack of education and instruction on cleanliness and food safety, street food vendors produced food in unhygienic settings.

Major recommendations to improve the situation:

1. **Improved Hygiene Practices:** Street food vendors and restaurant staff must receive training on proper food handling, hygiene, and sanitation practices to reduce the risk of microbial contamination.

2. **Regular Monitoring and Testing:** Establishing routine monitoring and testing programs for microbial hazards in food establishments can help identify and address potential issues promptly.
3. **Public Awareness Campaigns:** Educate the public about safe food consumption practices and encourage them to choose food vendors and restaurants that prioritize hygiene.
4. **Access to Safe Water:** A street food corners can be established with adequate supply of water and other utility services. Such centers will provide an environment for storing, preparing and serving safe food as well as ensure compliance with hygiene standards.

৯। Policy Recommendation

1. Appropriate and effective food vending structures, with potable water holding options, adequate light and separate waste basket holding capacity with desired standards should be made available in the market.
2. The street vendors must be given license- clear legal status so they are able to claim their entitlements to pursue their livelihoods. This can be done in coordination with the Ministries of Health (MOH) and Local Government by developing legislation to recognize the street food vendors.
3. The regulatory authority must develop food safety and hygiene training module and certification system, and make such training and certification compulsory before doing any food catering business.
4. Continued public awareness campaign along with periodic monitoring and testing program can help in improving the food safety and hygiene status.

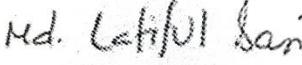
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The prevalence of pathogenic microorganisms including *E. coli*, *Salmonella* spp and *Vibrio* spp was evident in all the street food sample and salad samples analyzed. The presence of these three pathogens in food is not acceptable by the regulatory authorities because it may cause risk of infection to the public. The quantitative microbiological risk assessment of the all the street food and salad results revealed that highest risk of *E.coli* infection (45.5%) can be occurred through cholamuri consumption, followed by chatpoti (21.0%), sugarcane juice (4.0 %), aloe vera sharbat (4.0%), and negligible risk from mixed salad (0.00%) and sandwiches (0.00%). The risk of *Salmonella* infection is evident with aloe vera sharbat (10.5%),

followed by mixed salad (8.0%), and sugarcane juice (5.5%), while minimal or no risk of infection associated with *Salmonella* from cholamuri, sandwiches and chatpoti. The highest *Vibrio* spp infection can be occurred through consumption of chatpoti (22.0%) followed by sandwiches (14.5%), while minimal risk of infection associated with *Vibrio* is from aloevera sharbat, sugarcane juice, mixed salad and cholamuri. The study results depicted that the street food possess significant risk of the consumers and improvement food handlers hygiene and access to adequate safe water along with continuous monitoring is necessary to improve the situation. Nonetheless, more extensive analysis with more samples in conformity with the BfSA microbiological standards for RTE food products is required to finish this study.


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